Aurora C Kinase

#7384 Store at –80°C

☑5 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full-length human Aurora C (Met1-Ser275) kinase, supplied as a GST fusion protein.

Background: Aurora kinases belong to a highly conserved family of mitotic serine/threonine kinases with three members identified among mammals: Aurora A, Aurora B and Aurora C (1,2). Studies on the temporal expression pattern and subcellular localization of Aurora kinases in mitotic cells suggest an association with mitotic structure. Their functional influences span from G2 to cytokinesis and may be involved in key cell cycle events such as centrosome duplication, chromosome bi-orientation and segregation, cleavage furrow positioning and ingression (3). Aurora A is detected at the centrosomes, along mitotic spindle microtubules and in the cytoplasm of mitotically proliferating cells. Aurora A protein levels are low during G1 and S phases and peak during the G2/M phase of the cell cycle. Phosphorylation of Aurora A at Thr288 in its catalytic domain increases kinase activity. Aurora A is involved in centrosome separation, maturation and spindle assembly and stability. Expression of Aurora B protein also peaks during the G2/M phase of the cell cycle, while kinase activity peaks at the transition from metaphase to the end of mitosis. Aurora B associates with chromosomes during prophase prior to relocalizing to the spindle at anaphase. Aurora B regulates chromosome segregation through the control of microtubule-kinetochore attachment and cytokinesis. Expression of both Aurora A and Aurora B during the G2/M phase transition is tightly coordinated with histone H3 phosphorylation (4,5), while overexpression of both kinases is seen in a variety of human cancers (2,4). Aurora C localizes to the centrosome from anaphase to cytokinesis and both mRNA and protein levels peak during G2/M phase. Although typical Aurora C expression is limited to the testis, overexpression of Aurora C is detected in various cancer cell lines (6).

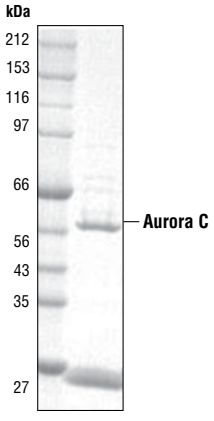

Figure 1. The purity of the GST-Aurora C protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human Aurora C (Met1-Ser275) (GenBank Accession No. NM_003160) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-Aurora C protein is 58 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Aurora C kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Warner, S.L. et al. (2003) *Mol. Cancer Ther.* 2, 589–595.

(2) Katayama , H. et al. (2003) *Cancer Metastasis Rev.* 22, 451–464.

(3) Andrews, P.D. et al. (2003) *Curr. Opin. Cell Biol.* 15, 672–683.

(4) Pascreau, G. et al. (2003) *Prog. Cell Cycle Res.* 5, 369–374.

(5) Crosio, C. et al. (2002) *Mol. Cell. Biol.* 22, 874–885.

(6) Kimura, M. et al. (1999) *J. Biol. Chem.* 274, 7334–7340.

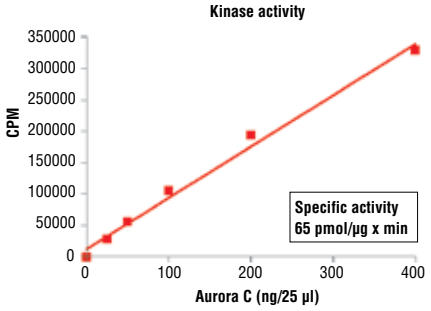

Figure 2. Aurora C kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, 50ng/µl BSA, Substrate: MBP 200 ng/µl, and variable amounts of recombinant Aurora C.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 150 mM NaCl, 0.25 mM DTT, 0.1mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at -80° C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

HTScan® Aurora C Kinase Assay Kit #7516

PLK (Ser137) Biotinylated Peptide #1300

Phospho-PLK (Ser137) Biotinylated Peptide #1597

Phospho-PLK (Ser137) Antibody #5070

Protocol for Aurora C Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Kinase Buffer (5X)**
   25 mM MOPS, pH 7.2
   12.5 mM β-glycerophosphate
   5 mM EGTA
   2 mM EDTA
   25 mM $MgC1_2$
   0.25 mM DTT
   250 ng/µl BSA
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. MBP (0.5 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer by 1:40 to make 250 µM ATP.
2. Dilute [^{32}P] ATP to 0.16 µCi/µl [^{32}P] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice
4. Dilute Aurora C protein (100 ng/µl concentration) to 40 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted Aurora C kinase solution, 10 µl MBP (0.5 µg/µl), and 5 µl 0.16 µCi/µl [^{32}P] ATP solution.

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
5 mM $MgCl_2$
0.05 mM DTT
50 ng/µl BSA
50 µM ATP
200 ng/µl MBP

6. After 15 minutes terminate reaction by spotting 20 µl of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.